HEALTH HOLDING

HAFER ALBATIN HEALTH CLUSTER MATERNITY AND CHILDREN HOSPITAL

Department:	Laboratory and Blood Bank (Haematology)		
Document:	Internal Policy and Procedure		
Title:	Mixing Studies - Screening for Inhibitor		
Applies To:	All Laboratory Staff		
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1. PURPOSE:

1.1 To establish system and set responsibilities for work.

1.2 To elucidate the accurate procedure for distinguishing between factor deficiency and circulating anticoagulant.

2. DEFINITONS:

N/A.

3. POLICY:

3.1 The accurate procedure for distinguishing between factor deficiencies and circulating anticoagulant.

3.2 When there is a prolongation of the PT or PTT, one should obtain a clinical diagnosis and medication history to explain the abnormality. Numerous disease and medication (e.g. warfarin, coumarin, heparin, streptokinase, and tissue plasminogen activator) may be the cause. If no such history to explain the results is found, one should perform a mixing study on fresh plasma to distinguish between inhibitor of a coagulation factor and a factor deficiency.

4. PROCEDURE:

4.1 Principle:

4.1.1 This involves mixing the patient plasma in a 1:1 ratio with pooled normal plasma and repeating the PT and/or PTT. If the clotting time corrects, i.e. returns to normal, the defect is a factor deficiency, which the pooled plasma has overcome by supplying the missing factor. If the clotting time does not correct, the defect is an anticoagulant, which acts equally on the pooled plasma and on the patient plasma, prolonging the clotting time.

4.2 Specimen Requirements & Processing

4.2.1 Obtain venous blood by clean venipuncture.

4.2.1.1 Immediately mix 9 parts blood with 1 part anticoagulant.

4.2.1.2 Mix well by inversion of the tube.

4.2.1.3 Centrifuge the specimen at 3500 rpm for 5 minutes

4.2.1.4 Remove the plasma within 60 minutes using a plastic pipette.

4.2.1.5 Store the plasma in a plastic tube.

4.2.1.6 Test the plasma sample within 2 hours, otherwise store frozen and thaw just prior to use.

4.2.1.7 Specimens without the proper amount of anticoagulant (i.e. short draws) or if haemolyzed should not be analyzed .Request a new sample.

4.2.2 Steps:

4.2.2.1 Mix 200uL of the abnormal patient plasma with 200uL of fresh normal Pool. If necessary, thaw the Pool rapidly at 37°C in the heat block. The total volume is not critical, but the ratio must be 1:1.

4.2.2.2 Place mixture on the specimen rack.
4.2.2.3 Run as a patient sample in PT/PTT mode on the BCS. This will give the results of mixing studies on both the PT and the PTT.
4.2.2.4 Compare the mixing test results to those of the undiluted sample to see if correction has occurred.
4.2.2.5 Show all specimen results to a hematology specialist physician (if possible). They will determine if a mixing study is indicated.
4.2.2.6 Perform a PT or APTT or both depending if the PT or APTT or both are elevated with the following normal pool, patient and control: patient mixture.

4.2.3 Calibration:
4.2.3.1 No calibration required

4.2.4 Quality control:
4.2.4.1 This is a standard PT / PTT run. Therefore all the Q.C. procedures applicable to the PT & PTT run apply to the mixing test: i.e. a Pool sample must be run with every PT & PTT run, and the Normal and Pathological Controls must be run once daily.

4.2.5 Procedure note:
4.2.5.1 Correction is defined as the conversion of the abnormal result (PT or PTT) to a normal result on mixing with normal Pool plasma.
4.2.5.2 All reagents must be fresh. This applies especially to the Pool sample used for the dilution, as it is the source of the coagulation factors required for correction.
4.2.5.3 Some inhibitors, e.g. Factor VIII inhibitor, do not act instantly, but act progressively over period of time. Hence the anticoagulant activity of a Factor VIII inhibitor will increase on incubation of the Pool/Patient mixture.

5. MATERIAL AND EQUIPMENT:

5.1 Reagents:
5.1.1 APTT or PT reagents as described in those procedures.

6. RESPONSIBILITIES:

6.1 The assigned technician

7. APPENDICES:

N/A

8. REFERENCES:

8.1 Harmening, D.M., Clinical Hematology and Fundamentals of Hemostasis. 2nd Ed. 1992, F.A.Davis Company
8.2 Hathaway, W.E., Goodnight, S.H., and Disorders of Homeostasis and Thrombosis: A Clinical Guide. 1st Ed. 1993, McGraw-Hill.

9. APPROVALS:

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